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Status of the Aflatoxin Problem in Corn

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ABSTRACT

Aflatoxin, a potent carcinogenic metabolite produced by the fungus *Aspergillus flavus*, has been clearly demonstrated to be a kernel contaminant in both preharvest and postharvest corn. Research has been conducted on: (i) fungus invasion of the ear; (ii) effects of the environment on aflatoxin levels; and (iii) methods of control.

A. flavus infects developing ears in both southern and corn belt regions but growing conditions in the southern U.S. are more conducive to the fungal infection process and subsequent toxin production. Most observations indicated that a break in the pericarp is necessary for fungal establishment. Insects, particularly larvae of the second European corn borer, corn earworm, and fall armyworm, have been implicated in causing breaks as well as serving as vehicles for carrying the inoculum to the potential infection site. Conditions of stress such as those caused by drought have been shown to enhance the aflatoxin contamination problem. Suggested control methods are: (i) plant only adapted hybrids with the highest level of resistance against ear-damaging insects; (ii) employ management practices that will either reduce stress or shift the ear development stage to miss a likely stress period.

Preliminary results suggest that either *A. flavus* growth or aflatoxin levels are under genetic control, but corn genotypes cannot be recommended at this time that would entirely eliminate the aflatoxin contamination problem in corn.

Additional Index Words: toxin, carcinogenic.

The death of 100,00 turkey poults in England in 1960 caused by a carcinogenic metabolite of *Aspergillus flavus*, later called *aflatoxin*, was traced to the protein supplement made from contaminated peanuts (7). During the subsequent 17 years the toxin has been intensively investigated. Currently it ranks as the most studied mycotoxin, probably due to its occurrence in food materials and demonstrated carcinogenic activity in laboratory animals (17).

During the 1960's aflatoxin contamination was clearly demonstrated in commercial cottonseeds, corn, copra, and treenuts, in addition to peanuts (19). Since most of these commodities or their by-products are frequently used in foods and feeds, scientists became concerned about developing methods for reducing levels of the toxin in commodities.

Prior to 1971, aflatoxin contamination in corn was believed to occur only in stored grain (3). After it was demonstrated that aflatoxin was found in preharvest corn (1), researchers launched numerous studies to answer questions associated with: (i) fungal invasion of the ear; (ii) effects of environment on aflatoxin levels; and (iii) methods of control.

One of the major impediments to studies of field occurrence of aflatoxin has been the sampling problem inherent in detection of a substance that occurs sporadically with a high degree of variation in level. To facilitate screening procedures, a simple, rapid, presumptive test was developed (11). The test was based on observations that linked a bright, greenish-yellow fluorescence (BGY) under ultraviolet light in cotton fibers to *A. flavus* growth. Investigation of the origin of the BGY-fluorescing material demonstrated that it was not aflatoxin but a derivative of kojic acid, another fungal metabolite. A similar fluorescence was observed in *A. flavus*-infected corn, and a procedure was developed for preliminary screening based on the fluorescent emission. BGY fluorescence has been widely used as a procedure for estimating the presence of aflatoxin in corn. However, the test is rather inconclusive, since a range of 25–70% incidence of chemically defined aflatoxin in BGY-fluorescing kernels has been observed (8, 11, 12). Therefore, the BGY method can best be used for preliminary screening, preferably on cracked kernels. Subsequent chemical analysis should be used for verification of aflatoxin presence and levels.

The objective of this report is to present the current status of research on the problem of aflatoxin in corn.

Initial Studies

Research reports by the end of 1974 concerning aflatoxin contamination in corn showed the following: (i) extensive *A. flavus* infection and aflatoxin accumulation occurs in preharvest corn, especially in certain production areas of the United States (1, 2, 6, 8, 9, 11, 13, 16, 18); (ii) *A. flavus* infects developing corn ears both in southern and corn belt-grown corn, but growing conditions in the southern areas of the United States appear to be more conducive to the fungal infection process and subsequent toxin production (10, 14); (iii) developing kernels are most vulnerable to infection during the late-milk/early-dough stage, but infection can also occur at later stages of maturation (10, 16); (iv) the stages of grain maturity are an important determinant of infection by the fungus (10, 13, 14); (v) most observations indicate a break in the pericarp tissue is needed for establishment of the fungus (10, 16); (vi) stress conditions such as those caused by drought may predispose the plant to attack by a number of pests, including *A. flavus* (10); (vii) insects, particularly larvae of the second generation European corn borer, corn earworm, fall armyworm, and some grain-storage insects can cause kernel pericarp breaks that are potential sites for *A. flavus* infection (4, 10, 21, 22, 23); (viii) certain ear-damaging insects serve as a vehicle for carrying the fungal inoculum to the developing kernels (4, 10); (ix) preliminary results suggest that certain corn genotypes may possess genetic factors for either resistance to *A. flavus* fungal growth or lower aflatoxin levels (12); and (x) accurate estimates of aflatoxin contamination are complicated by low incidence levels, sampling requirements, and the sporadic nature of the contamination.

Infection Process

Although insects have been implicated in *A. flavus* infection in preharvest corn, several aspects of the infection process remain ambiguous and require further study including: (i) availability of the inoculum (source and level); (ii) method of spore transmission to the infection site; (iii) establishment of initial infection by the fungus in developing kernels; and (iv) distribution of the secondary inoculum from the original infection site to other noninfected kernels of the ear.

Inoculum Availability

Availability of *A. flavus* fungal inoculum was determined in silks of developing ears from identical corn genotypes grown at several locations in the United States. The fungus was recovered from silks in at least 7 of 48 samples collected in Missouri, Illinois, Iowa, South Carolina, Georgia, and Texas (5). Incidence of the fungus was higher in silks from mature ears than from developing ears. The *A. flavus* spores found in silks might have been the result of: (i) fall-out of airborne spores, or (ii) insect activity transporting fungal conidia to the silks from soil, decaying vegetation, or infected stored grain. *A. flavus* inoculum potential would be a very important factor in the development of an early warning system designed to forecast an aflatoxin problem in developing corn.

Fungal-Spore Insect Relationship

The dispersal of fungal inoculum was studied by examining the internal fungi of insects collected from corn ears at test sites located in Georgia, South Carolina, Texas, Missouri, Illinois, and Iowa (5). From these six locations, 3,442 insects were collected, classified, and examined. The incidence of internal *A. flavus* spores among larvae and adults of stored-grain insects, European corn borer, corn earworm, and other insects was relatively uniform, with a range from 1.7 to 3.1%. *A. flavus* was found in insects from five of the six locations. The fungus was not observed in insect samples from Iowa. However, that may have been due to the low number of insects (53) obtained at that site. The incidence of *A. flavus* was relatively low in insects at both 30 days postflowering and at maturity but significantly higher for the later date.

Of the 3,442 test insects, 87 had ingested the fungal propagules prior to removal from ears. Although internal presence of the fungus would not be proof for dissemination, it would represent a distinct advantage for infection of developing kernels by *A. flavus*, since frass from these insects should provide an ideal medium and environment for spore germination and fungal growth.

The presence of *A. flavus* fungal spores internally in insects collected from wide geographical areas raises the question of detrimental effects of the fungus on the insect. The interregional study suggested that *A. flavus* toxicity to insect hosts was related to aflatoxin produced by the fungus (5). Since only one-third of the fungal isolates obtained from insects produced aflatoxin, the insect-borne fungal strains were clearly heterogeneous for toxin production. The results from these studies demonstrated a complex interrelationship between insects, fungi, and kernel infection.

Further Documented Field Occurrence of Aflatoxin

Prior to 1975, numerous field investigations showed that *A. flavus* can infect corn before harvest (1, 16, 20) but showed only limited evidence of the fungus occurring in preharvest corn in the central and northern regions. In the fall of 1975, the news media carried numerous accounts of BGY fluorescence in Iowa corn (8, 9). Since prior information suggested extensive *A. flavus* infection might be restricted to southern corn growing regions of the United States, a study was conducted to determine the extent of *A. flavus* infection and aflatoxin contamination in the 1975 Iowa corn crop in the field. Samples were collected from six test areas in a three-county region of west-central Iowa and a centrally located county. Samples were collected during harvest directly from the picker-sheller or receiving trucks. Of the 214 samples collected, 101 or 47% showed BGY fluorescence and 37 or 17% had aflatoxin. No statistical evidence was found for difference in either BGY or toxin occurrence between the six test areas but wide dif-

ferences in the level of infection were noted among fields sampled. Of the 37 toxin-positive samples, only 4 had aflatoxin B_1 levels exceeding 20 ppb: the highest was 56 ppb. Mycological studies identified the presence of *A. flavus*-infected kernels in 60% of 214 samples.

Visual examination of preharvest ears in western Iowa showed characteristic greenish-yellow spores of *A. flavus* on some ears, and when these ears were analyzed they had a range in aflatoxin from 1 to 1,560 ppb. However, randomly selected ears without visible evidence of *A. flavus* infection also had some aflatoxin.

In almost all instances, ears with visible evidence of *A. flavus* infection had insect damage caused by second generation European corn borer larvae (9). However, not all ears damaged by European corn borer were infected by *A. flavus*. The possible secondary spread of *A. flavus* infection to kernels adjacent to an insect-damaged infection site was examined and none was found.

Differences in the regional distribution of BGY fluorescence in the 1975 corn crop in Iowa was observed; the southwest region had the most frequent occurrence, whereas little or no fluorescence was observed in corn grown in the northeast region. Examination of the climatological records for these two regions showed less rainfall (26 June–9 August: 0.5 inch in the southwest vs. 1 to 2 inches in the northeast) and higher average temperatures for the southwest region. The southwest region had 41 days in July and 36 days in August with temperatures above 32.2°C (90°C), compared with 21 in July and 10 in August for northeast Iowa. This observation substantiates previous observations that drought stress during the silk-to-dough stage of kernel development is an environmental factor that favors the fungal infection process.

Although extensive *A. flavus* infection and BGY fluorescence were noted in the 1975 Iowa corn crop, only limited incidence of aflatoxin at relatively low levels was found. However, the results of the Iowa sampling study showed that corn belt corn is not immune to the preharvest *A. flavus* infection provided a favorable environment and kernel damaging insects are prevalent.

1977 Aflatoxin Problem

In August 1977, numerous reports were obtained from corn production areas in the southestern U.S. on the extensive occurrence of *A. flavus* infection and aflatoxin contamination in corn grain both in preharvest and harvested condition (G. Meyer. Aflatoxin found in southern corn puts dealers, government on alert. *Wall Street Journal*, 20 Sept. 1977. p. 32). Preliminary observation indicated more than 90% of the corn crop was contaminated with the toxin. Aflatoxin levels were unusually high, with many samples exceeding 1,000 ppb (personal communication from D. Wilson, Univ. of Georgia, Tifton). The high level of aflatoxin and *A. flavus* infection was attributed to the extreme stress conditions brought about by the worst drought experienced in many years. The drought also enhanced the numbers and activity of corn-earworm and fall army-worm larvae. The insects appeared early and inflicted heavy damage throughout the growing season.

Reports of aflatoxin in 1977 were received from Iowa, where certain regions, especially central Iowa, experienced the most severe drought on record. The Iowa State Chemist's office reported that of 87 samples of corn harvested in the field, 16 contained more than 20 ppb aflatoxin (J. Dickinson. Elevator operators holding the bag on corn crop. *Ames Daily Tribune*, 27 Sept. 1977. p. 1). Most of the aflatoxin-positive samples came from the drought-stressed areas. Subsequently, other corn belt locations reported samples with BGY fluorescence in the 1977 crop (R. Rothballer. Possibly poisonous fungus found in stored area corn. *Peoria Journal Star*, 24 Sept. 1977), but chemical tests showed little or no aflatoxin (D. Lane. Aflatoxin test results near. *Peoria Journal Star*, 27 Sept. 1977. p. B-4).

Control

STRESS

Plant stress occurring especially during the silking to late dough kernel development stage appears to enhance *A. flavus* infection and subsequent production of aflatoxin. Since 1971, in almost all instances aflatoxin-contaminated preharvest corn occurred in areas that had experienced severe drought stress. These observations indicate that any action to ease stress conditions during the silking to dough stage should alleviate the degree of infection by *A. flavus* and the level of aflatoxin. Early planting in most corn production areas moves the silking dough-development stage ahead to miss the likely stress period that is often encountered by later planted corn. By proper choice of planting date, periods of stress during the critical grain filling period can be avoided.

Corn hybrids differ in their response to stress. Although it has not been definitely demonstrated that hybrids with increased stress resistance have reduced amounts of aflatoxin, it would seem a reasonable assumption.

Cultural practices that would alleviate stress include irrigation, weed control, and adequate soil fertility. The selection and planting of adapted hybrids is an essential control measure since nonadapted hybrids are not likely to endure stress as well as the adapted ones.

INSECT CONTROL

Earworm—Since kernel-damaging insects have been shown as likely vectors of *A. flavus* inoculum, and kernel pericarp injury is the site for infection and eventual penetration of the fungus, reduction of insect damage should be reflected in less aflatoxin. Corn earworm damage differs greatly among corn hybrids. Most of the hybrids adapted to the southern regions of the United States have higher levels of resistance to corn earworm than hybrids adapted to the corn belt. The resistance is largely morphological, characterized by long, tight husks, whereas the corn belt hybrids tend to have shorter loose husks for facilitation of rapid ear-moisture drying in the field.

Earworm can also be controlled by spraying with insecticides. A study carried out in South Carolina and Florida spraying Seven (1-naphthyl-N-methylcarba-

mate) on developing corn ears three times/week (1-1.5 lb/acre) for a 6-week period after silking showed that the treatment significantly reduced earworm damage and aflatoxin levels at maturity in both locations (12). In a similar study in Georgia, Gardona [2-chloro-1-(2,4,5-triethylchlorophenyl)vinyl dimethyl phosphate] was applied to ears three times/week (0.75 lb/acre) during a 6-week period following silking. Insecticide treatment reduced mean earworm damage about 60% and aflatoxin 50% (13). Although corn earworn damage can be reduced by spraying with an insecticide and subsequent lower aflatoxin levels in mature corn, the number of applications necessary almost eliminates this method of control from an economic viewpoint.

Second Generation European Corn Borer—During recent years the second generation European corn borer has become a major pest of corn in the corn belt. In the period 1940-1960 the first generation European corn borer was very destructive, but damage by this pest has been greatly reduced by incorporating resistance factors into the commonly grown hybrids (2). At first, sources of resistance were difficult to locate, but once they were located and after it was established they were quantitatively inherited, the level of resistance was rapidly increased in source populations through cyclic selection. Sources of resistance to the second generation have been more difficult to locate and it is practically nonexistent in corn belt-adapted material. With the lack of genetic resistance in currently grown hybrids, the losses from this insect have reached significant economic levels. Since larval feeding of the second generation European corn borer occurs during the time kernels are developing, the insect becomes a major suspect in the dissemination of *A. flavus* fungal inoculum in corn grown in the midwestern U.S. and in other areas where the insect is found.

Fall Armyworm—The effort of breeding resistance to the fall armyworm has not been extensive and very little resistance has been identified. Losses from this insect can become large in some years. Since larvae of this insect damage the ear in any stage of growth, it is a potential causal agent of *A. flavus* transfer to developing kernels.

GENETIC CONTROL

Limited research has been conducted to determine whether inherited differences in aflatoxin levels exist among corn genotypes. The first studies showed significant differences between two hybrids. Hybrid I adapted to the corn belt had higher aflatoxin levels than Hybrid II adapted to the southern U.S. (14) when grown at four locations in the United States and inoculated with the same strain of *A. flavus*. The southern locations, Georgia and Texas, had significantly higher aflatoxin levels than Illinois and Missouri. In another study, the comparisons among short-season hybrids grown in South Carolina showed significant differences in aflatoxin levels that ranged from 89 to 245 ppb (15). A second study conducted in South Carolina and Florida, with five single crosses adapted to South Carolina and one hybrid adapted to the corn belt, showed highly significant differences among hybrids for

BGY fluorescence and aflatoxin levels (12). The corn belt had much higher aflatoxin levels than the five South Carolina hybrids. One South Carolina single cross had the lowest aflatoxin level when grown in South Carolina and in Florida.

Significant differences among hybrids were found for aflatoxin levels as the result of either natural or artificial inoculation. However, verification of the results required repetition in consecutive crops. In most studies of field occurrence, aflatoxin variation has been extremely high; this diversity has been attributed to inadequate sampling and nonuniform inoculation techniques.

INHERITANCE STUDY

To determine a genetic roll in the production of aflatoxin in preharvest corn, eight inbred parental lines were chosen with no prior evaluation for either *A. flavus* fungal growth response or aflatoxin production (24). The eight inbred lines were crossed to give the 28 possible single crosses and 28 reciprocals for a total of 56 crosses. Ears were inoculated 20 days after flowering date with a pinboard that pierced the pericarp of 6 rows of 20 kernels in length. Inoculum of *A. flavus* was sprayed directly on the injured kernels. Ears were harvested at physiological maturity and infected kernels were bulked on a plot basis.

Differences in aflatoxin levels among single crosses were significant but not for reciprocal crosses. Data were subjected to a diallel analysis and estimates of general combing ability (GCA) effects were obtained. Parental lines H60 and Mo17 had large negative effects; N104, N28, H84, and N7B had intermediate effects; and Mo5 and Oh545 had large positive GCA. Aflatoxin levels were lowest for crosses between low by low parents and highest for high by high parents. Crosses of low by high had aflatoxin levels approaching the high parent, suggesting dominance of the high parent. The results from this study indicated that aflatoxin levels were under genetic control; however, more research is needed to substantiate these findings.

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